

REGULATION OF TRANSENDOTHELIAL LOW-DENSITY
LIPOPROTEIN TRANSMISSION BY FLUID SHEAR STRESS

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Abstract

The link between atherosclerosis and regions of disturbed flow and low wall shear stress is now firmly established, but the causal mechanisms underlying the link are not yet understood. A crucial factor in the early stages of the disease is the blood-to-tissue transport of low-density lipoprotein (LDL). It is well known that a high level of LDL in the blood is a risk factor for atherosclerosis, but the relationship between flow characteristics and LDL transport are not well understood. In this paper, we use a previously derived similarity solution to obtain the concentration profile of LDL at the endothelium, in an arbitrary vessel geometry, in terms of the wall shear stress. The transendothelial transport of LDL is then evaluated downstream of a backward-facing step. It is found that the LDL concentration changes very little over the region of interest and that the main factor in determining the rate of intimal accumulation of LDL is endothelial macromolecular permeability. It is shown that a previously hypothesised dependence of permeability on the spatial gradient of wall shear stress is not necessary to produce higher LDL accumulation in the region of disturbed flow. Experimental data are required to elucidate the precise relationship between wall shear stress and endothelial permeability and the signalling mechanisms involved.

Key terms: arterial intima, atherosclerosis, cholesterol, endothelium, permeability, viscous flow.

1 Introduction

The progressive narrowing and hardening of the arteries, known as atherosclerosis, and associated complications such as thrombosis, heart attack and stroke, are the leading cause of death in the developed world. The pathogenesis of atherosclerosis, in which lipoproteins, fibrous substances and inflammatory cells accumulate in the arterial wall has received considerable attention in recent decades¹⁹.

The disease begins with elevated levels of cholesterol-carrying low-density lipoprotein (LDL) in the layer of the vessel wall known as the intima. This layer is separated from the bloodstream by a monolayer of endothelial cells (EC). In the intima, LDL is oxidised and induces expression by EC and intimal smooth muscle cells (SMC) of a range of adhesion molecules and chemotactic factors responsible for the adhesion of leukocytes to the endothelium and their subsequent penetration into the intima¹⁰. Additional EC- and SMC-derived chemokines stimulate differentiation of leukocytes into cholesterol-scavenging macrophages, and subsequent macrophage proliferation and expression of LDL receptors. The macrophages engulf LDL and become foam cells, forming a fatty streak in the intima, the characteristic feature of early atherosclerotic lesions¹³. The accumulation of foam cells in the intima, the first stage of atherosclerosis, is referred to as intimal hyperplasia (IH).

IH is exacerbated by the continued proliferation of SMC in the intima and recruitment of SMC from the sub-intimal layer of the arterial wall known as the media. Activated SMC synthesise extracellular matrix (ECM) components, such as fibronectin and collagen, which increase the size of lesion and may form a fibrous membrane or ‘cap’ around the atheroma. Persistent presence of LDL in the bloodstream allows prolonged growth of the plaque, which may continue for several years. In advanced stages of atheroma, which is now a complex mixture of LDL, oxidised LDL, leukocytes, SMC and matrix proteins, macrophages may start to secrete matrix metalloproteases (MMPs) that digest ECM proteins in the plaque and its fibrous cap¹². The plaque may subsequently rupture, often leading to thrombosis and marking the transition from the chronic to the acute,

and potentially fatal, stage of atherosclerosis.

There are a variety of well known environmental risk factors, including smoking, high-cholesterol diet and lack of exercise, that increase the likelihood of atherosclerosis. Moreover, it is now established that IH does not occur randomly throughout the circulatory system, but occurs much more frequently near arterial bifurcations and in areas of high vessel curvature⁸. IH is strongly associated with disturbed haemodynamic characteristics, such as flow detachment from the arterial wall, flow recirculation and oscillatory flow. IH is particularly well correlated with areas of the vasculature that experience low time-averaged wall shear stress¹⁴. The causal link between low wall shear stress and the onset of IH has long been the subject of investigation, but has yet to be fully elucidated.

It has become increasingly recognised that the endothelium is not simply a passive barrier, but plays a crucial role in maintaining vascular homeostasis and regulating the passage of materials between the blood and the vessel wall²⁴. EC are sensitive to mechanical stimuli such as wall shear stress, as well as biochemical stimuli such as blood-borne agonists. EC are also a source of numerous vasoactive factors, such as nitric oxide (NO), prostaglandin, endothelin and angiotensin¹⁹.

In linear sections of the vasculature with steady laminar flow and relatively high wall shear stress (greater than 1 Pa), EC are well aligned in the direction of flow and cell-cell junctions are tight⁸. As a consequence, the endothelium is a highly stable cell population, with a very low rates of proliferation and death⁵, and low permeability to blood-borne macromolecules, such as LDL¹⁹. EC synthesise a number of vasoactive substances, including the potent vasodilators, NO and prostaglandin¹⁴. These act in a paracrine manner on SMC in the intima, resulting in relaxation of the arterial wall. Plaque formation in these areas is rare.

In contrast, areas of disturbed flow and low wall shear stress are often associated with a poorly aligned and disorganised endothelium⁵. Endothelial turnover and permeability are increased, resulting in enhanced transmission of atherogenic matter into the intima and thus increasing likelihood of IH²⁴. Synthesis of NO

is reduced, whilst expression of vasoconstrictors and mitogenic factors, such as endothelin and platelet-derived growth factor, are upregulated, increasing the rate of SMC proliferation and protein synthesis in the intima, which accelerates the process of plaque formation¹⁴.

The flow characteristics, in addition to determining the shear stress to which the EC are exposed, affect the delivery of blood-borne molecules to the endothelium⁶. These include adenosine nucleotides and thrombin, which are capable of eliciting a response in EC via binding to G-protein-coupled surface receptors. The mechanism by which the EC detect and respond to the prevailing haemodynamic conditions and the delivery of biochemical stimuli is unclear, but is almost certainly linked to intracellular calcium (Ca^{2+}) signalling. Ca^{2+} is a key intracellular second messenger responsible for regulating a variety of cellular functions²³.

John and Barakat¹¹ formulated a model for the mass transport of adenosine triphosphate (ATP) and diphosphate (ADP) in a parallel plate vessel, including the effects of nucleotide uptake and production by EC, and considered both steady and pulsatile flow. David³ extended this model to derive an analytical solution for the concentrations of ATP and ADP in an arbitrary vessel geometry with a known shear stress profile. This is particularly important from our point of view as it allows the effects of different vessel geometries, a key factor in the occurrence of atherosclerosis, to be examined. In Plank *et al.*¹⁷, the solution of David³ was used to simulate ATP uptake at the endothelium and the resultant intracellular Ca^{2+} signalling. The relationship between wall shear stress and platelet adhesion and aggregation, important factors in thrombus formation, have also been modelled⁴.

In this paper, we investigate the link between wall shear stress and atherogenesis by using the solution of David³ to calculate the rate of flux of LDL across the endothelium in different areas of the blood vessel. It is thought that wall shear stress affects endothelial permeability to macromolecules such as LDL, but this effect has not been fully characterised and the underlying mechanisms are unknown²⁴. One consequence of application of fluid flow to EC is the alignment and elongation of cells in the direction of flow due to the mechanical shear stress

to which they are exposed¹. It is thus possible that shear stress helps the EC to organise into a confluent monolayer with tight gap junctions, a low rate of turnover and low macromolecular permeability². In contrast, areas of the endothelium exposed to low or oscillatory shear stress are poorly aligned with a high rate of EC proliferation, and may therefore exhibit higher macromolecular permeability^{5,7}. It would thus appear that permeability is a decreasing function of wall shear stress.

Stangeby and Ethier²² performed a computational analysis of blood-wall arterial transport of LDL, using a vessel geometry representing an artery partially occluded by atherosclerotic plaque. Their model included LDL transport in the endothelium and the arterial intima, and permeability to LDL was taken to be an increasing function of wall shear stress. The effect of the haemodynamic conditions of the growth and progression of the plaque was examined.

Here, we are concerned with the initial stages of atherogenesis, in which enhanced transendothelial LDL transport leads to the development of a fatty streak in the arterial intima. We therefore use an unstenosed geometry, representing a healthy vessel. Previous modelling work on mass transport in the vasculature has focussed on the parallel plate geometry, with uniform wall shear stress^{21,11}. In order to investigate directly the relationship between haemodynamic conditions and atherogenesis, we use the backward-facing step, representing a widening of the blood vessel, as a model geometry (see Figure 1). The advantage of this geometry is that the fluid flow has non-uniform wall shear stress, a recirculation region and a stagnation point, key features of areas associated with atherosclerosis *in vivo*.

Interaction between the endothelium and the macromolecule dextran in a backward-facing step flow chamber has been studied experimentally¹⁶ and it was observed that macromolecular transport across the endothelium was enhanced in the recirculation zone. It was concluded that the mechanism responsible for the differential behaviour in the recirculation zone was not the absolute value of wall shear stress, but its spatial gradient. However, it is not clear whether individual EC are capable of detecting such a gradient or what mechanisms underly the cellular response. Furthermore, it is unknown whether enhanced transendothe-

lial transport in the recirculation zone was due to increased permeability of the endothelial layer, or to more efficient mass transport of macromolecules to the endothelial surface. These are issues we aim to investigate in this modelling study.

2 Model for Mass Transport

In this section, we describe a model for the mass transport of chemical species in the bloodstream. The model assumes steady flow, but is quite general in the sense that it can be applied to any chemical species with a low diffusion coefficient, and any vessel geometry with a known steady wall shear stress profile (see David³ for details). Atherosclerosis, at least in the developmental stages, is a chronic disease in which the lesion grows over a period of years. In contrast, the cardiac cycle has a time-scale of approximately one second, so the assumption of steady flow is justified.

Figure 1 shows the model setup, which consists of a two-dimensional rigid tube. The side $y = 0$ downstream of the step constitutes a reactive surface, representing a monolayer of endothelial cells (EC). For a given vessel geometry with a known steady flow profile, $(u(x, y), v(x, y))$, with characteristic velocity, u_∞ , the concentration, $\phi_i(x, y)$, of chemical species i with diffusion coefficient D_i obeys the following advection–diffusion equation in the bulk fluid:

$$u \frac{\partial \phi_i}{\partial x} + v \frac{\partial \phi_i}{\partial y} = D_i \nabla^2 \phi_i. \quad (1)$$

Uptake and production of the species occurs on the reactive surface, $y = 0$, representing the endothelium. We therefore have the following boundary condition on $y = 0$:

$$D_i \frac{\partial \phi_i}{\partial y}(x, 0) = V_i(x) \phi_i(x, 0) - S_i(x), \quad (2)$$

where $V_i > 0$ is a constant. This states that the rate of uptake is proportional to its concentration at the endothelium and the rate of production is equal to $S_i(x)$.

The species concentration at the point where medium enters the vessel, $x = x_1$,

is a fixed value, $\phi_{i,\infty}$, and the concentration is assumed to approach this value a long way from the endothelium (i.e. as $y \rightarrow \infty$):

$$\phi_i(x_1, y) = \lim_{y \rightarrow \infty} \phi_i(x, y) = \phi_{i,\infty}. \quad (3)$$

2.1 Non-Dimensionalisation

We denote the wall shear stress by $\tau_w(x)$ and define the following dimensionless quantities:

$$x' = \frac{x}{L}, \quad y' = \frac{y}{L}, \quad u' = \frac{u}{u_0}, \quad v' = \frac{v}{u_0}, \quad \phi'_i = \frac{\phi_i}{\phi_0}, \quad \tau'_w = \frac{\tau_w}{\rho u_0^2},$$

$$Re = \frac{u_0 L}{\nu}, \quad Pe = \frac{u_0 L}{D_0}, \quad D'_i = \frac{D_i}{D_0}, \quad \phi'_{i,\infty} = \frac{\phi_{i,\infty}}{\phi_0}, \quad V'_i = \frac{V_i L}{D_0}, \quad S'_i = \frac{S_i L}{D_0 \phi_0},$$

where ν and ρ are respectively the kinematic viscosity and density of blood.

An order of magnitude analysis reveals that $\frac{\partial^2 \phi_i}{\partial y^2} \gg \frac{\partial^2 \phi_i}{\partial x^2}$, so diffusion in the x -direction may be neglected and the advection-diffusion equation (1), on dropping the dashes, becomes:

$$u \frac{\partial \phi_i}{\partial x} + v \frac{\partial \phi_i}{\partial y} = \frac{D_i}{Pe} \frac{\partial^2 \phi_i}{\partial y^2}. \quad (4)$$

The boundary conditions (2) and (3) become:

$$D_i \frac{\partial \phi_i}{\partial y}(x, 0) = V_i(x) \phi_i(x, 0) - S_i(x), \quad (5)$$

$$\phi_i(x_1, y) = \lim_{y \rightarrow \infty} \phi_i(x, y) = \phi_{i,\infty}. \quad (6)$$

2.2 Similarity Solution

For many chemical species of interest, such as adenosine nucleotides and macromolecules, the diffusion coefficient, D_i , in the bloodstream is very low and therefore the Peclet number, Pe , is very high. As a result, the mass transfer boundary layer at the endothelium, $y = 0$, is much thinner than the velocity boundary layer and we may assume that the streamwise velocity, u , is linear with respect to y in the mass transfer layer.

In such cases, it has been shown⁴ that if a similarity variable, η , is defined by:

$$\eta = y \frac{(Pe Re)^{\frac{1}{3}} |\tau_w(x)|^{\frac{1}{2}}}{\left(9 \operatorname{sgn}(x - x_0) \int_{x_0}^x |\tau_w(s)|^{\frac{1}{2}} ds\right)^{\frac{1}{3}}} \equiv y \beta(x), \quad (7)$$

then the governing equation (4) for chemical concentration may be written as the following ordinary differential equation (ODE):

$$D_i \frac{d^2 \phi}{d\eta^2} + 3\eta^2 \frac{d\phi}{d\eta} = 0. \quad (8)$$

The solution of this ODE satisfies

$$\phi(\eta) = A_i \int_0^\eta \exp\left(-\frac{s^3}{D_i}\right) ds + \phi_i(x, 0).$$

The boundary condition at the endothelium (5) demands that

$$A_i = \frac{1}{D_i \beta(x)} (V_i(x) \phi_i(x, 0) - S_i(x)).$$

Noting that $\phi_i(\eta) \rightarrow A_i D_i^{\frac{1}{3}} \frac{\Gamma}{3} + \phi_i(x, 0)$ as $\eta \rightarrow \infty$ (where $\frac{\Gamma}{3} = \int_0^\infty e^{-t} t^{-\frac{2}{3}} dt$), the concentration at the endothelium, $\phi_i(x, 0)$, may be found by imposing the boundary condition (6) at $\eta = \infty$. Hence the solution at $y = 0$ may be written:

$$\phi_i(x, 0) = \frac{\phi_{i,\infty} \beta(x) D_i^{\frac{2}{3}} + \frac{\Gamma}{3} S_i(x)}{\beta(x) D_i^{\frac{2}{3}} + \frac{\Gamma}{3} V_i(x)}. \quad (9)$$

The function β defined by (7) has a singularity at $x = 0$. In the case $\tau_w(0) \neq 0$, $\beta(x) \rightarrow \infty$ as $x \rightarrow 0$, which means that $\lim_{x \rightarrow 0} \phi_i(x, 0) = \phi_\infty$. In the case $\tau_w(0) = 0$, Taylor expanding $\tau_w(x)$ about $x = 0$ yields $\lim_{x \rightarrow 0} \beta(x) = \left(\frac{Pe Re \frac{d\tau_w}{dx}(0)}{6} \right)^{\frac{1}{3}}$.

2.3 Model for LDL

Let us now consider a specific chemical species of particular relevance to the development of atherosclerosis: low-density lipoprotein (LDL), a cholesterol-carrying molecule. LDL is a key player in atherogenesis: exposure of EC to excessive levels of LDL results in the endothelial injury that is characteristic of the early stages of disease; the transendothelial transport of LDL is the major contributor to the development of a fatty streak¹⁰. As is widely known, a high level of LDL in the blood is one the main risk factors associated with atherosclerosis. An LDL concentration in the blood of less than 2600 μM is considered

desirable, whilst above this level, the risk of developing atherosclerotic plaque increases. Clearly the interplay between LDL concentration at the endothelium and fluid shear stress is of great importance to the link between low shear stress and incidence of atherosclerosis.

The main source of LDL in the bloodstream occurs as a result of the digestive process in specialised regions of the circulatory system. As a result, there is no production of LDL in the section of vessel under examination in this model: $S_{LDL}(x) = 0$.

The permeability of the endothelium to LDL is highly variable and depends on a range of factors. In particular, it is thought that permeability is an increasing function of the local LDL concentration⁹ and is also regulated by wall shear stress. Although there is some evidence to suggest that permeability is enhanced in areas of low wall shear stress^{24,7}, the mechanism by which shear stress exerts this effect is unknown. It is likely that intracellular Ca^{2+} signalling is involved as Ca^{2+} is known to play a key role in agonist-mediated increases in endothelial permeability^{15,18}. On the other hand, some authors have reported that permeability *increases* with wall shear stress²², whilst others claim that it is not the absolute value of wall shear stress that is important, but the spatial gradient of wall shear stress¹⁶.

We believe that, until more data are available regarding the role of Ca^{2+} in shear stress-mediated regulation of permeability, it would be somewhat speculative to construct a detailed mathematical model of this process. We therefore adopt a phenomenological approach and use a simple functional relationship between shear stress and permeability, without making any assumptions about the precise mechanisms involved. In this way, we can easily test the potential effects of different relationships between shear stress and endothelial permeability. This will pave the way for more detailed future modelling of the intracellular signalling pathways via which wall shear stress modulates EC phenotype.

Note that the permeability of the endothelium to macromolecules is very low (of the order $10^{-10} \text{ m s}^{-1}$ ²⁵) and it may be seen from equation (9) that the LDL concentration will change very little over the section of vessel being modelled. To

avoid introducing unnecessary unknowns into the model, we will therefore ignore the dependence of permeability on LDL concentration and take the permeability, V_{LDL} , to be a function only of the local wall shear stress. We hence take

$$V_{\text{LDL}}(x) = V_0 \frac{|\tau_w(x)| + a_1}{|\tau_w(x)| + a_2}, \quad (10)$$

where $V_0 > 0$ and $a_1, a_2 > 0$ are constants. The form of this function means that V_{LDL} is bounded above and bounded away from zero. If $a_1 > a_2$ then V_{LDL} is a decreasing function of $|\tau_w|$, whereas if $a_2 > a_1$ then V_{LDL} is an increasing function of $|\tau_w|$. We will examine both these cases and the functions used are shown in Figure 2.

We will also examine the case where V_{LDL} is a function of the shear stress gradient, $|\frac{d\tau_w}{dx}|$, by taking

$$V_{\text{LDL}}(x) = V_0 \frac{\left|\frac{d\tau_w}{dx}(x)\right| + b_1}{\left|\frac{d\tau_w}{dx}(x)\right| + b_2}, \quad (11)$$

where $b_2 > b_1 > 0$ are constants.

The parameter values used in the model are shown in Table 1. For a discussion of these values, see the Appendix.

3 Results

We now compute the LDL concentration using equation (9), and the transendothelial LDL flux using the boundary condition (5), as functions of the distance downstream of a backward-facing step. We first study the case where the permeability of the endothelium to LDL is an decreasing function of absolute wall shear stress (see equation (10) and Figure 2). Figure 3 shows the wall shear stress as a function of x and Figure 4 shows the LDL concentration profile. Because the endothelial permeability is very low (regardless of the relationship with shear stress, its maximum value is $2 \times 10^{-9} \text{ m s}^{-1}$), the rate of uptake of LDL at the endothelium is small compared to the bulk fluid concentration and there is only a very small drop in concentration over the region of interest. This justifies our decision to neglect the dependence of endothelial permeability on LDL concentration.

Figure 5 shows the transendothelial LDL flux, which is much higher in the recirculation zone than in the area of fully developed flow further downstream. This is because the wall shear stress is lower in this area (in particular, wall shear stress is zero at the reattachment point at $x = 0$ and at the point at the base of the step at $x = -1.64$), so permeability is higher, enhancing transport of LDL across the endothelium. Although the actual rate of flux is small (of the order $10^{-6} \mu\text{M m s}^{-1}$), LDL accumulation in the intima can take place over a period of many years before reaching dangerous levels. Thus the difference in flux between different parts of the vessel shown in Figure 5 could be sufficient to localise the formation of a fatty streak to the recirculation zone. This is in agreement with observations that atherosclerosis occurs almost exclusively in regions of disturbed flow and low wall shear stress²⁴.

Figure 6 shows the transendothelial LDL flux in the case where permeability is an *increasing* function of wall shear stress (see equation (10) and Figure 2). This time, the situation is reversed in that the LDL flux is higher in the region of fully developed flow than in the recirculation zone. This is unsurprising since wall shear stress, and hence permeability, is higher in the developed flow region, allowing a greater rate of LDL flux across the endothelium. This result appears to contradict the observed pattern of occurrence of atherosclerosis *in vivo*.

Finally, Figure 7 shows the transendothelial LDL flux in the case where permeability is an increasing function of the spatial *gradient* of wall shear stress (11). The results are similar to the case where permeability is a decreasing function of absolute wall shear stress (Figure 5): LDL flux is higher in and around the recirculation zone than in the fully developed flow area. This is in agreement with the experimental data of Phelps and DePaola¹⁶, who observed that the accumulation of the macromolecule dextran was highest in the recirculation zone, where the spatial wall shear stress gradient is high.

4 Discussion

A similarity solution for chemical species concentration in the bloodstream³ has been used to calculate the concentration of the atherogenic macromolecule LDL at the endothelium downstream of a backward-facing step. It was found that, because of the low permeability of the endothelium, LDL concentration changed very little (less than 1%) over the region studied (approximately 2.5 mm of blood vessel downstream of an arterial widening). It therefore appears that, although the bulk fluid concentration of LDL is clearly a key factor in determining the risk of atherosclerosis, the LDL concentration at the endothelium *relative to the bulk fluid* is a much less important factor than the permeability of the endothelial monolayer to LDL. The rate transport of LDL from the bloodstream into the intima has been calculated for three qualitatively different relationships between wall shear stress and macromolecular permeability of the endothelium. These were:

- (i) permeability decreases with increasing wall shear stress;
- (ii) permeability increases with increasing wall shear stress;
- (iii) permeability increases with increasing wall shear stress *gradient*.

The results for (ii) showed a higher tendency for intimal LDL accumulation in the region of fully developed flow than in the region of disturbed flow. This is contrary to the vast majority of clinical observations of sites of atherosclerotic plaque, which localise preferentially to regions of disturbed flow characteristics and low or oscillatory wall shear stress.

The results for the (i) and (iii) were similar and predicted higher intimal LDL accumulation in the recirculation zone adjacent to the backward-facing step, a finding in agreement with clinical observations. The fact that these two relationships produced such similar results suggests that a dependence of endothelial permeability on the spatial gradient of wall shear stress, as hypothesised by Phelps and DePaola¹⁶, is not necessary to produce elevated levels of intimal LDL accumulation in the recirculation zone. This effect can be produced by

the simpler mechanism in which endothelial permeability is high when absolute wall shear stress is low and vice versa. This is a more plausible mechanism from a cellular point of view since high wall shear stress promotes EC alignment and gap junction formation^{2,24}, whereas low wall shear stress allows EC proliferation and disrupts barrier function⁵.

The fact that LDL concentration changes very little over the region of interest means that differences endothelial permeability, as opposed to differences in mass transport to the endothelium, must play an important role in regulating blood-to-tissue transfer of LDL. A wide range of local and systemic risk factors contributes to atherogenesis²⁰ and it is an advantage of this modelling approach that the contribution to atherogenic events of different factors, such as mass transport of macromolecules in the bloodstream and changes in endothelial permeability, can be separately evaluated. Mathematical modelling thus complements experimental studies, in which, for example, quantification of abluminal macromolecular accumulation measures the aggregate atherogenic effect, but often cannot distinguish the relative importance of the different factors involved¹⁶. On the basis of the results of this model, we hypothesise that low wall shear stress contributes to atherogenesis by enhancing endothelial permeability to macromolecules and thus allowed accelerated transport of LDL into the arterial intima. Clearly this is a simplification and a number of other factors are involved, but it would appear that this shear stress-dependent regulation of LDL transport is of central importance in the early stages of disease as it provides a direct contribution to formation of a fatty streak.

More experimental data are needed firstly to confirm or refute the proposed inverse relationship between permeability and wall shear stress, and secondly to elucidate the mechanisms involved. These will almost certainly involve intracellular signalling, with Ca^{2+} signalling a likely candidate because of the known dependence of agonist-dependent regulation of endothelial permeability on intracellular Ca^{2+} ^{15,18}. A model of endothelial Ca^{2+} signalling in response to fluid shear stress and agonist delivery was formulated in¹⁷, based on the similarity solution of David³ and the calcium dynamics model of Wiesner *et al.*^{27,26}. An important target for future work is to couple the effects of the Ca^{2+} sig-

nalling process to phenotypic characteristics related to atherogenesis. It is likely that cytosolic Ca^{2+} concentration plays a modulating role, sensitising the cell to other stimuli such as growth factors and other vasoactive agents. Formulation of a model of the role of endothelial Ca^{2+} signalling in atherogenesis will therefore require careful thought as to which extracellular stimuli need to be included.

A limitation of the similarity solution for chemical species concentration is that it can only predict the effects of steady flow on EC and macromolecular mass transport. This is justifiable for the present model, in which a simple phenomenological relationship between shear stress and permeability is assumed, because gross accumulation of LDL in the intima takes place over many years, whereas the timescale for pulsatile flow associated with the cardiac cycle is of the order of one second. However, in a more detailed future model that includes the intracellular signalling pathway, it will be important to include the effects of pulsatile flow. This is because the resting (i.e. steady state) concentration of cytosolic Ca^{2+} is less important than *changes* in the cytosolic Ca^{2+} concentration. A time-dependent model will be necessary to capture such behaviour and this will be addressed in a future paper.

Appendix

Bulk LDL concentration. The American Heart Association describes an LDL concentration of less than $2600\text{ }\mu\text{M}$ as ‘optimal’, $2600\text{--}3300\text{ }\mu\text{M}$ as ‘near-optimal’, $3300\text{--}4100\text{ }\mu\text{M}$ as ‘borderline high’ and over $4100\text{ }\mu\text{M}$ as ‘high’. We used the value of $\phi_{\text{LDL},\infty} = 3000\text{ }\mu\text{M}$ as representing an average, healthy adult. The effects of high levels of LDL in the bloodstream could be easily investigated by increasing this parameter.

Basal LDL permeability and diffusivity. Truskey *et al.*²⁵ measured endothelial permeability to LDL as $2 \times 10^{-10}\text{ m s}^{-1}$ and we choose the value of V_0 in equations (10), (10) to be such that the *minimum* permeability is $2 \times 10^{-10}\text{ m s}^{-1}$. Stangeby and Ethier²² gave the LDL diffusion coefficient in blood as $5 \times 10^{-12}\text{ m}^2\text{s}^{-1}$.

LDL permeability parameters. These are unknown and a number of functional forms were tested with a range of parameter values. The values $a_1 = 40$, $a_2 = 4$ for the decreasing case and $a_1 = \frac{5}{9}$, $a_2 = \frac{50}{9}$ for the increasing case give a maximum permeability that is 10 times the minimum permeability, and half-maximal permeability at a wall shear stress of $|\tau_w| = 5$ (equivalent to approximately 0.54 Pa). Similarly, the values $b_1 = \frac{20}{9}$, $b_2 = \frac{200}{9}$ for the gradient-dependent case gives half-maximal permeability at a shear stress gradient of $|\frac{d\tau_w}{dx}| = 20$ (equivalent to approximately 8.68 Pa mm⁻¹). Changing the values of these parameters changes the sensitivity of the endothelial permeability, V_{LDL} , to the wall shear stress (gradient), but does not qualitatively change the transendothelial LDL flux profile.

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Dimensional values	
Bulk LDL concentration	$\phi_{\text{LDL},\infty} = 3000 \mu\text{M}$
LDL basal uptake rate	$V_0 = 2 \times 10^{-10} \text{ m s}^{-1}$
LDL diffusion coefficient	$D_{\text{LDL}} = 5 \times 10^{-12} \text{ m}^2\text{s}^{-1}$
Step height	$h = 2.5 \times 10^{-4} \text{ m}$
Reference lengthscale	$L = 2.5 \times 10^{-4} \text{ m}$
Reference concentration	$\phi_0 = 3000 \mu\text{M}$
Reference velocity	$u_0 = 1.04 \times 10^{-2} \text{ m s}^{-1}$
Reference diffusion coefficient	$D_0 = 5 \times 10^{-12} \text{ m}^2\text{s}^{-1}$
Kinematic viscosity of blood	$\nu = 4 \times 10^{-6} \text{ m}^2\text{s}^{-1}$
Density of blood	$\rho = 1000 \text{ kg m}^{-3}$
Dimensionless values	
Reynolds number	$Re = 0.651$
Peclet number	$Pe = 5.2 \times 10^5$
Bulk LDL concentration	$\phi_{\text{LDL},\infty} = 1$
LDL basal uptake rate	$V_0 = 0.01$
LDL diffusion coefficient	$D_{\text{LDL}} = 1$

Table 1: Parameter values used in the model for LDL mass transport.

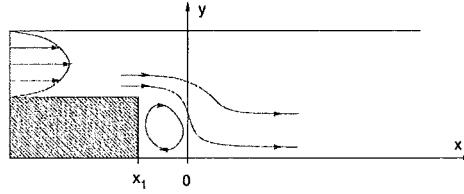


Figure 1: Diagram of the backward-facing step geometry, representing a widening of the artery. The flow detaches from the endothelium at $x = x_1$ and reattaches at $x = x_0 = 0$.

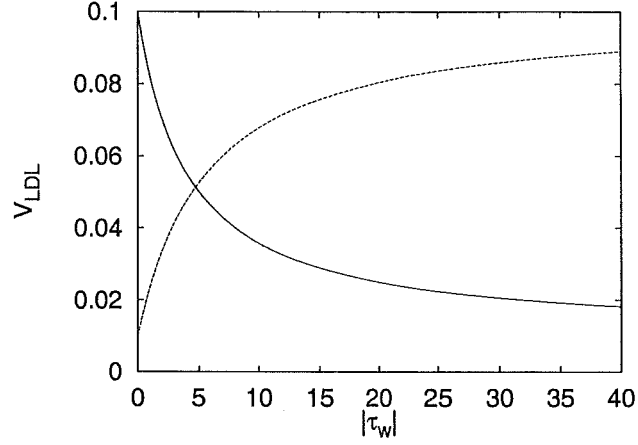


Figure 2: Endothelial permeability to LDL, V_{LDL} , as a function of absolute wall shear stress, $|\tau_w|$. For the decreasing function, $V_0 = 0.01$, $a_1 = 40$, $a_2 = 4$; for the increasing function, $V_0 = 0.1$, $a_1 = \frac{5}{9}$, $a_2 = \frac{50}{9}$. Plotted values are dimensionless: to obtain dimensional values, V_{LDL} should be multiplied by $2 \times 10^{-8} \text{ m s}^{-1}$ and τ_w by 0.108 Pa.

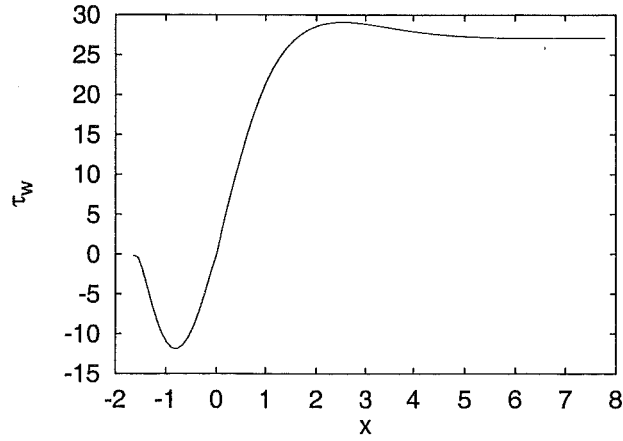


Figure 3: Graph of wall shear stress, $\tau_w(x)$, against x . The origin ($x = 0$) is taken to be at the reattachment point (where $\tau_w = 0$). To obtain dimensional values, τ_w should be multiplied by 0.108 Pa and x by $2.5 \times 10^{-4} \text{ m}$.

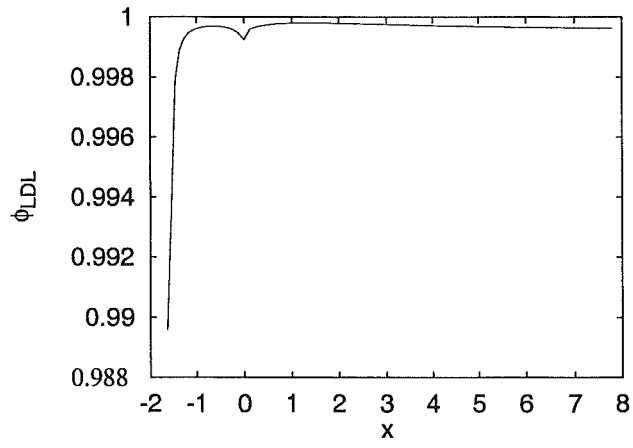


Figure 4: Graph of the LDL concentration, ϕ_{LDL} against x where permeability is a decreasing function of shear stress. Note there is very little change in LDL concentration over the region of interest. To obtain dimensional values, ϕ_{LDL} should be multiplied by $3000 \mu\text{M}$ and x by $2.5 \times 10^{-4} \text{ m}$.

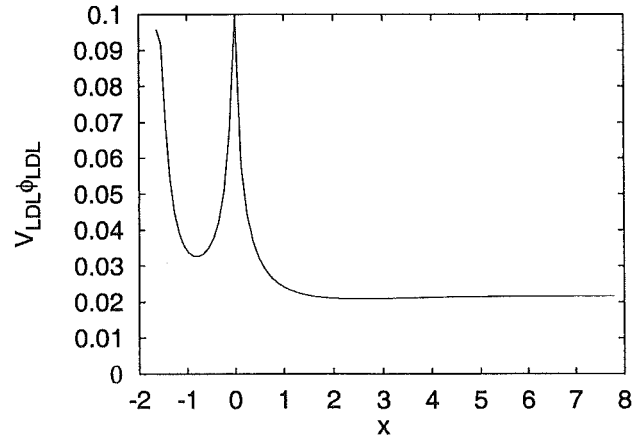


Figure 5: Graph of the transendothelial LDL flux, $V_{\text{LDL}}\phi_{\text{LDL}}$ against x where permeability is a decreasing function of shear stress. LDL flux is higher in and near to the recirculation zone ($x < 0$) than in the region of fully developed flow downstream ($x > 2$). Values used: $V_0 = 0.01$, $a_1 = 40$, $a_2 = 4$. To obtain dimensional values, $V_{\text{LDL}}\phi_{\text{LDL}}$ should be multiplied by $6 \times 10^{-5} \mu\text{M m s}^{-1}$ and x by $2.5 \times 10^{-4} \text{ m}$.

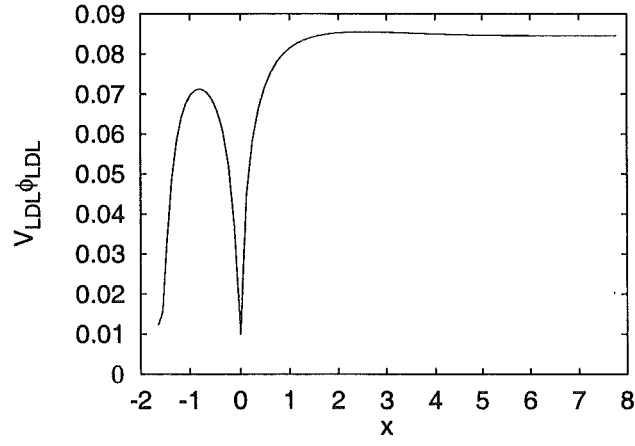


Figure 6: Graph of the transendothelial LDL flux, $V_{LDL}\phi_{LDL}$ against x where permeability is an increasing function of shear stress. LDL flux is higher in the region of fully developed flow downstream ($x > 2$) than in the recirculation zone ($x < 0$). Values used: $V_0 = 0.1$, $a_1 = \frac{5}{9}$, $a_2 = \frac{50}{9}$. To obtain dimensional values, $V_{LDL}\phi_{LDL}$ should be multiplied by $6 \times 10^{-5} \mu\text{M m s}^{-1}$ and x by $2.5 \times 10^{-4} \text{ m}$.

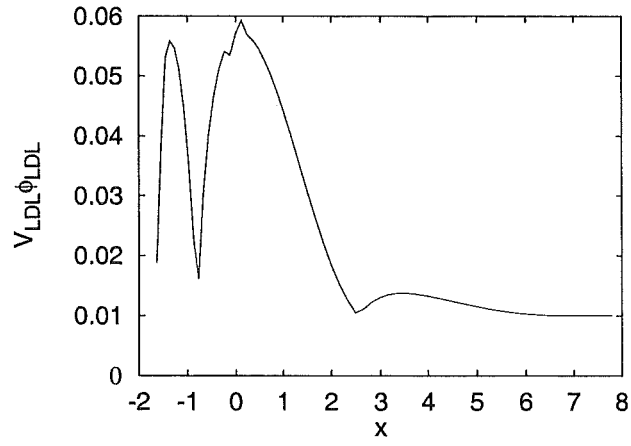


Figure 7: Graph of the transendothelial LDL flux, $V_{LDL}\phi_{LDL}$ against x where permeability is an increasing function of shear stress gradient. LDL flux is higher in and near to the recirculation zone ($x < 0$) than in the region of fully developed flow downstream ($x > 2$). Values used: $V_0 = 0.1$, $a_1 = \frac{20}{9}$, $a_2 = \frac{200}{9}$. To obtain dimensional values, $V_{LDL}\phi_{LDL}$ should be multiplied by $6 \times 10^{-5} \mu\text{M m s}^{-1}$ and x by $2.5 \times 10^{-4} \text{ m}$.